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Magnetic resonance imaging for non-invasive measurement of plastic ingestion in marine wildlife

Kathryn E. Anderssen [a,*], Geir Wing Gabrielsen [b], Mathias Kranz [c,d], France Collard [b]

[a] Department of Seafood Industry, Nofima AS, P.O. Box 6122, 9291 Tromsø, Norway
[b] Norwegian Polar Institute, Fram Centre, 9296 Tromsø, Norway
[c] PET Imaging Center Tromsø, University Hospital North-Norway (UNN), 9009 Tromsø, Norway
[d] Nuclear Medicine and Radiation Biology Research Group, The Arctic University of Norway, UiT, 9009 Tromsø, Norway

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ABSTRACT

Monitoring plastic ingestion by marine wildlife is important for both characterizing the extent of plastic pollution in the environment and understanding its effect on species and ecosystems. Current methods to detect plastic in the digestive system of animals are slow and invasive, such that the number of animals that can be screened is limited. In this article, magnetic resonance imaging (MRI) is investigated as a possible technology to perform rapid, non-invasive detection of plastic ingestion. Standard MRI methods were able to directly measure one type of plastic in a fulmar stomach and another type was able to be indirectly detected. In addition to MRI, other standard nuclear magnetic resonance (NMR) measurements were made. Different types of plastic were tested, and distinctive NMR signal characteristics were found in common for each type, allowing them to be distinguished from one another. The NMR results indicate specialized MRI sequences could be used to directly image several types of plastic. Although current commercial MRI technology is not suitable for field use, existing single-sided MRI research systems could be adapted for use outside the laboratory and become an important tool for future monitoring of wild animals.

1. Introduction

Marine plastic pollution is a rapidly growing environmental problem (Savoca et al., 2021; MacLeod et al., 2021). In addition to the risk of entanglement (Nelms et al., 2015) and suffocation (Andrades et al., 2021), the ingestion of plastic (Azevedo-Santos et al., 2019) poses a significant threat for marine wildlife. For example, plastic ingestion by seabirds has been detected across the globe and approximately half of the world's seabird species are believed to contain plastics in their stomachs (Wilcox et al., 2015). A study of 555 fish species found ingested plastic in two-thirds of them, including 210 species that are important for commercial fishing (Savoca et al., 2021). Biofilms that grow on the surface of plastic in the ocean can mimic the smell of food, encouraging its consumption by marine animals (Savoca et al., 2016; Savoca et al., 2021). Plastic ingestion can cause internal wounds or blockages in the digestive system (Gregory, 2009), as well as adversely affect growth rate, body mass and quality of life due to decreased stomach capacity (Yin et al., 2018; Chae et al., 2019; Collard and Ask, 2021). Consequently, plastic pollution is already having a negative effect on fish stocks around the globe (Weerakoon and Grøsvik, 2019) and is becoming a leading cause of death in many seabird species (Roman et al., 2019). Plastic pollution in the ocean is also known to collect toxic chemicals (e.g. polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochlorine pesticides) and heavy metals (Rochman et al., 2019) that then have the potential to transfer to muscle, liver and brain tissue upon ingestion (Rochman et al., 2013; Sun et al., 2019). Recent research has found strong evidence for the transfer of polybrominated diphenyl ether from ingested plastic into the tissue of northern fulmars (Kühn et al., 2020; Neumann et al., 2021). Polybrominated diphenyl ether has been shown to increase in the brain when animals are losing body fat (Sagerup et al., 2009). However, although plastic ingestion can be deleterious for an individual animal, it is currently difficult to say how much of an effect it has on species at a population level (Dehnhard et al., 2019; Dias et al., 2019). Therefore, this highlights the need for more research to better understand the overall level and extent of threat that plastic pollution poses to wildlife.

In order to fight plastic pollution in the ocean, it is necessary to know where it is and where it is coming from (Ryan et al., 2009). Among the

* Corresponding author.
E-mail address: kate.anderssen@nofima.no (K.E. Anderssen).

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biggest challenges in determining the extent and effect of plastic ingestion by marine wildlife is its detection. Research into the effect of plastic ingestion in seabirds is hindered by the need for more screening (Baak et al., 2021). Of 20,000 marine fish species, only approximately 2 % have been tested for plastic ingestion so far (Savoca et al., 2021). At the moment, two main options exist for evaluating the presence of plastic in the stomach of marine wildlife. The first is to sacrifice the animal and then dissect it to investigate the stomach. This is wasteful and limits the number of animals that can be investigated. This can be problematic if the species in question happens to be endangered. It is also a heartbreaking aspect of the research, that in some situations, it is necessary for scientists to kill animals of a species to do the investigations meant to protect them. The second is stomach flushing (Provencher et al., 2021). This involves sending a large volume of water down into the stomach of the animal so they can regurgitate the stomach contents (Wilson, 1984; Ryan and Jackson, 1986). This is an unpleasant, stressful experience for the animal and there is uncertainty as to how much of the stomach contents have been produced. For example, a narrowing between the proventricular and the gizzard of fulmars does not allow regurgitation of the entirety of the stomach contents (van Franeker and Law, 2015). Unfortunately, in many situations, using found carcasses is not a feasible alternative. In remote locations such as Svalbard, collecting enough samples is not practical and predators quickly devour animal remains. Furthermore, accurate methods for estimating plastic ingestion in a population require a random sampling of its members. As high plastic consumption can lead to increased mortality, using beached birds will create skewed statistical distributions (Rodríguez et al., 2018; Collard et al., 2022).

In addition to providing information to understand its effect on species and ecosystems, tracking plastic ingestion is an important proxy for estimating the extent of plastic pollution in the environment. In the northern hemisphere, fulmars are well suited for biomonitoring trends in marine plastic pollution, as they are commonly recorded with much higher amounts of ingested plastics than other species of seabird (van Franeker et al., 2011; Trevail et al., 2015; Acampora et al., 2016). Fulmars are likely to ingest plastic items (van Franeker and Meijboom, 2002; van Franeker et al., 2011) due to their unselective surface-feeding behaviour (Azzarello and Vleet, 1987; Moser and Lee, 1992; Tourinho et al., 2010). They are well-suited as large-scale marine biomonitors because they only consume marine prey, have a limited capacity of regurgitation, and have wide migration ranges across the Barents Sea, the Greenland Sea, and the Labrador Sea (Falk and Møller, 1995; Weimerskirch et al., 2001). Because of this, the fulmar is included in the Oslo-Paris Convention (OSPAR) and the Arctic Monitoring and Assessment Program (AMAP) as an indicator species for monitoring of plastic in the sea (OSPAR Commission, 2008; AMAP, 2021). As well as knowing the amount of plastic consumed by animals, knowing the polymer type of plastic is also important. Different types of plastic tend to come from different sources. Consumer waste tends to consist of polyethylene (PE) or polyethylene terephthalate (PET) (PlasticsEurope, 2020) while waste from the fishing industry tends to be lines, ropes or threads made of polyamide, polypropylene, or polyethylene (Skvorčinskienė et al., 2019). By understanding where the plastic waste in a region likely stems from, steps can be taken to prevent future pollution.

In this article, we investigate the feasibility of magnetic resonance imaging (MRI) as a method for non-invasively measuring plastic ingestion in wildlife. MRI is a specialized application of nuclear magnetic resonance (NMR) (Callaghan, 1991; Bushong and Clarke, 2014). Despite the name, no harmful ionizing radiation is used in the technique. The technology uses a strong magnetic field to align the magnetic moment of nuclei in a sample. Strong radio frequency (rf) pulses are used to excite the system away from equilibrium and can be used to measure a wide variety of properties about a system. An advantage of the technique is that measurements can be made inside the sample, such that unwanted influence of other constituents, such as feathers or muscle, can be avoided. This overcomes a problem of other characterization techniques, such as hyperspectral imaging, where light is unable to penetrate deeply enough into the sample (Jacques, 2013).

MRI is the most well-known application of NMR, due to its increasingly common use in medicine, but in addition to imaging, NMR can also be used to provide information on the structure and chemistry of materials. Two commonly used NMR characterization techniques are the one-dimensional ^{1}H spectrum and the T_2 relaxation. The ^{1}H spectrum gives details about the chemical environment of hydrogen atoms in the sample. In plastics, due to the rigid structure, finer details of the chemical spectra are not able to be resolved. However, the width and pattern of the line shape still provides information on the chemistry. The T_2 relaxation is the time it takes the system comes to equilibrium among itself after excitation with an rf pulse and provides information on the physical structure. In this study, we use MRI images, the ^{1}H spectrum and T_2 relaxation distribution of plastics to evaluate MRI and NMR as methods for detecting and characterizing plastic ingestion in marine wildlife, represented by the northern fulmar (*Fulmarus glacialis* Linnaeus, 1761) hereafter called simply "fulmar". To this aim, there are three main questions to be answered. First, is MRI imaging of plastic possible? Although previous research was unable to image plastic using MRI (Ingraham et al., 2015), we anticipated that imaging should be possible with an MRI sequence optimized for materials with short T_2. Secondly, is distinguishing between plastic types possible using T_2 relaxation? To date, structural investigations of plastic by NMR have been limited to high-field, solid state studies (Dadayli et al., 1994; Eckman et al., 1997), which utilize expensive equipment that is not able to be adapted to measure on wildlife. In contrast, equipment to perform T_2 relaxation measurements is low cost and can be adapted to measure on wildlife. Based on the different physical structures, we expected the T_2 distributions from different plastic types will be sufficiently distinct to distinguish them. Finally, what equipment specifications would be necessary in order to use MRI technology as a routine method for screening for plastic ingestion? Based on the data to be collected, we expected that it should be possible to provide guidelines for what MRI equipment specifications would be required in order to both successfully image and identify different sorts of plastics.

2. Materials and methods

2.1. NMR and MRI measurements

Although NMR and its specialized application MRI are very versatile techniques, it is not possible to have hardware that can do every type of measurement. Tradeoffs must be made in the physical specifications depending on the intended application of the equipment, as having hardware optimized for one type of measurement will preclude the ability to do other types of measurements. As such, different types of NMR and MRI equipment are suited for different purposes and there exist many different configurations of hardware. In this study, two types of NMR systems were used. Firstly, a small animal MRI imager was used. This equipment has the capability to perform imaging on samples. However, due to its focus on medical research and soft tissue, it is not possible to measure signal from samples with extremely short relaxation times. As it is expected that plastics would have a significant portion of their signal at short relaxation times, a second NMR system was used. This system is designed for materials research, such that extremely signal from short relaxation can be measured, but it is limited to very small samples sizes and cannot perform imaging. There exist MRI systems with the capability to image samples with very short relaxation times, but none were practically available for this study.

2.1.1. MRI equipment and acquisition

MRI images were acquired using a preclinical 7 Tesla MR Scanner (MRS*DRYMAG, MR solutions, Guildford, UK) using a rat quadrature coil. Both T_2-weighted and T_1-weighted images were obtained. The T_2-weighted images were acquired with a standard fast spin echo sequence using a single average, an echo time of 45 ms, a repetition time of 8000 ms and 1 mm slice thickness. Field of view dimensions were 60 mm × 60 mm with a 250-μm pixel resolution. The T_1-weighted images were acquired with a standard fast spin echo sequence using a single average, an echo time of 11 ms, a repetition time of 1500 ms and 1 mm slice thickness. Field of view dimensions were 60 mm × 60 mm with a 250-μm pixel resolution. These sequences were chosen as they were the best available on the scanner for measuring upon samples with short T_2 relaxation values.

2.1.2. NMR equipment and acquisition

Benchtop NMR measurements were made using a Spinsolve 43 Mhz Spectrometer (Magritek Ltd., Aachen Germany). ^{1}H measurements were made with a single 90° pulse of length 12 μs, a dwell time of 1 μs, 16,384 points and a repetition time of 2000 ms. T_2 measurements were made with an echo spacing of 80 μs, a pulse length of 12 μs and a repetition time of 2000 ms. 2000 echoes were measured. A minimum signal to noise ratio of 200 was obtained for all measurements. This is well above 100 SNR, which is considered the rule of thumb cutoff to ensure that signal noise does not influence the T_2 distributions.

2.1.3. NMR data analysis

T_2 distributions were analysed using the inbuild inverse Laplace transform in the SpinSolve software. ^{1}H spectra were Fourier transformed and a Gaussian filter applied to smooth noise. Statistical analysis on the processed NMR results was performed in the R Programming language (Free Software Foundation Inc., Boston, MA). A principal component analysis (PCA) (Martens and Næs, 1992) was performed to evaluate clustering and similarity between the different plastic polymer types. The PCA is a linear axis transformation that converts the data into a new coordinate system where the axes are determined by the greatest variance in the data. ^{1}H spectra and T_2 distributions were normalized, centered, and scaled before PCA analysis.

2.2. Samples

2.2.1. Fulmar stomachs

MRI measurements were tested using four stomachs from deceased fulmar *(Fulmarus glacialis)* specimens. Fulmars are the species recommended for monitoring by the Arctic Monitoring and Assessment Program (AMAP, 2021) due to their surface feeding habits. Due to size limitations of the available coils, fulmar stomachs (both the gizzard and the proventriculus) were excised before measurement. Two of the stomachs were left empty. The other two stomachs were artificially stuffed with plastic (Fig. 1). Two types of plastic were used: pieces of a LDPE plastic bag and pipette tips of polypropylene. These are two of the most common types of plastic waste found in seabirds (Robuck et al., 2022). All samples were vacuum packed for hygiene reasons before MRI measurement using a pressure of 95 % vacuum to limit distortion and compression in the tissue.

2.2.2. Plastic samples

In addition to the fulmar stomachs, samples of polyethylene terephthalate (PET, Type 1), high-density polyethylene (HDPE, Type 2), low-density polyethylene (LDPE, Type 4) and polypropylene (PP, Type 5) were measured using a bench top NMR spectrometer. Examples of 5 of each type of plastic were tested. Samples were placed in 5 mm tubes for measurement (Fig. 2).

The PET plastic samples consisted of drink bottles and food packaging. The HDPE plastic samples consisted of shampoo, detergent, and

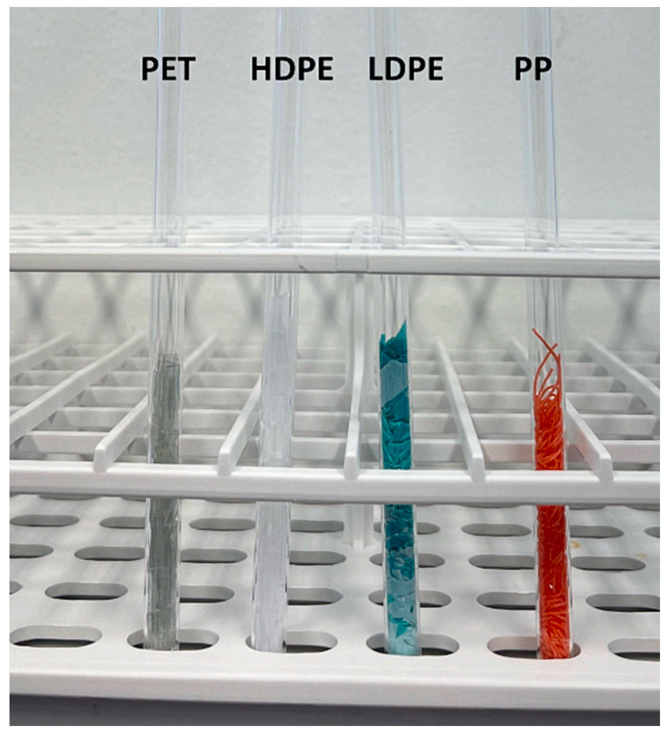

Fig. 2. Examples of PET, HDPE, LDPE and PP used for T_2 and ^{1}H measurement.

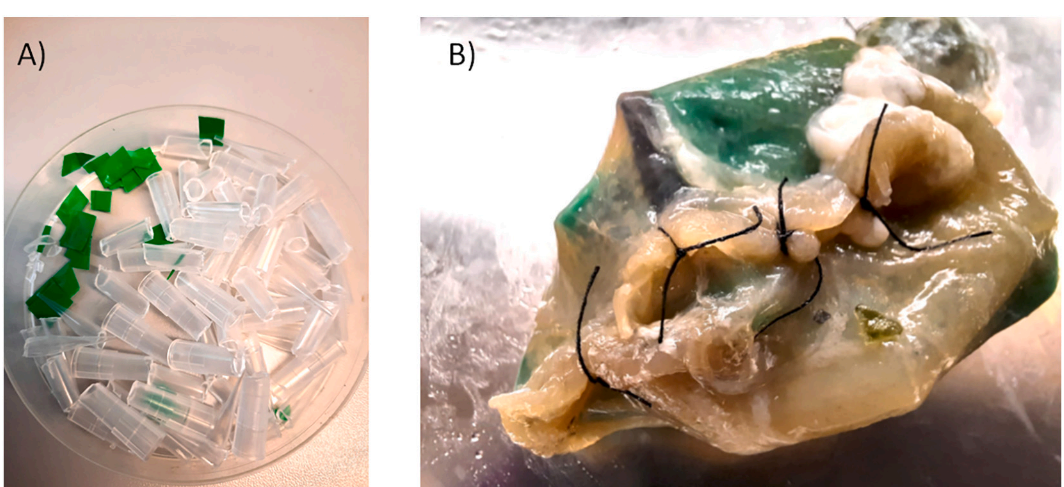

Fig. 1. A) Plastics used for testing B) Fulmar stomach artificially stuffed with plastic.

household cleaning chemical bottles. LDPE plastic samples consisted of a variety of plastic bags. Polypropylene consisted of pipette tips, food packaging as well as two sets of fishing rope. Polyvinyl chlorine (PVC, Type 3) was not tested due to its low prevalence in seabirds (e.g. Kühn et al., 2021; Neumann et al., 2021). Polystyrene (Type 6) was not tested because the large percentage of air per unit volume causes the signal from typical examples to be extremely weak. Five examples of each type of plastic were measured in order to test variability across the plastic type.

3. Results

The results of the study indicate that imaging of plastics and identification of polymer type possible is possible using NMR but will likely require the development of specialized equipment in order to perform the measurements in wildlife. Fig. 3a shows an image of an empty fulmar stomach and Fig. 3b one that contains plastic. No significant difference was seen in the T_1 versus T_2 weighted images. In the image, the polypropylene pipette tips are directly visible. In contrast, the LDPE plastic bag appears as void space in the stomach. Although it cannot be directly seen, its presence can be inferred by the deformation to the stomach.

The T_2 distributions and ^{1}H spectra from the bench top measurement of different plastic polymers are shown in Fig. 4. Location and relative intensity of T_2 peaks tended to be consistent within a plastic polymer type. This is witnessed by the clear clustering of the T_2 distributions and ^{1}H spectra for the different plastic polymers, shown Fig. 5. Distinct clustering is seen for both types of measurements, indicating that basic NMR measurements can be used to distinguish between the different polymers of plastic.

Note, due to the relaxation time of plastic being a Gaussian decay and on the order of the echo time, the reconstructed signal at short T_2 times can be artificially high. This is a well-established artefact (Washburn, 2014). PET has a strong peak at approximately 80 μs and two very weak peaks at 10 ms and 100 ms. HDPE has a strong peak at approximately 80 μs and a smaller peak around 700 to 800 μs. The peaks of HDPE tend to be broader than the PET and merge together. LDPE plastic is very similar in form to HDPE, with a strong peak at 110 μs and a weaker peak at 1 ms. PP plastic is similar in form to PET in that the majority of the signal is contained in a single, strong peak, though it also has a weak peak at approximately 1.5 ms, as well as sometimes weak peaks at tens of milliseconds. For the ^{1}H spectra, clear distinctions are also seen between the plastic polymer types. LDPE has a single, narrow peak while HDPE has a single broader peak. This is in line with expected behaviour given that the HDPE is a more rigid material than the LDPE. The PP peak is a clear overlay of a narrow peak and a broader peak. Interestingly, the PET shows a distinct triplet behaviour.

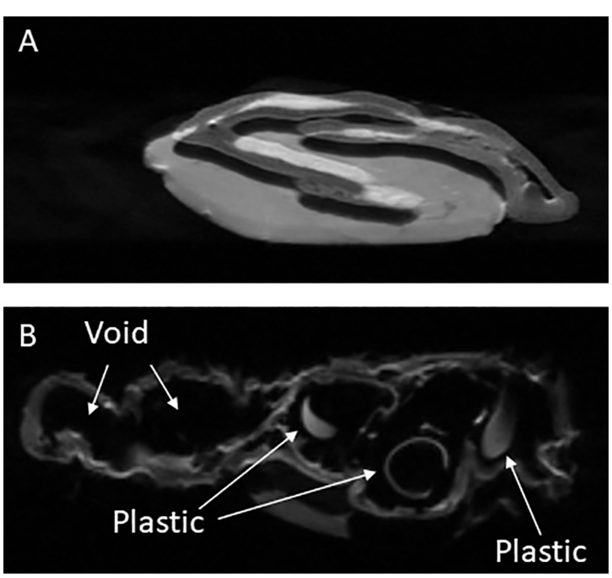

Fig. 3. T_2 images of A) empty fulmar stomach B) fulmar stomach containing plastic.

4. Discussion

The polypropylene plastic could be easily identified in the MRI images of the fulmar stomachs and, based off the results of the T_2 measurements, it is expected the other common types of should be possible to image as well with MRI equipment optimized for measurement of short T_2 samples. Although the stomachs were removed from the animals due to size restrictions of the available MRI equipment, this is not a restriction in general and whole birds or other animals (e.g. fish, turtles, etc.) could be measured with suitable equipment. An unexpected result was that the stiffer polypropylene was visible in the image while the more flexible LDPE was not, as T2 times are generally shorter in more rigid materials. Based on the T_2 relaxation results, it appears that there are often weak peaks at longer T_2 times in polypropylene samples, which must be the portion of the signal that was imaged. Although not currently possible on the available MRI system, there are imaging sequences that are designed for materials with extremely short relaxation that should be able to allow direct imaging of the LDPE and the other plastic polymers themselves (Pauly et al., 1989; Robson et al., 2003). With that in mind, even if plastic is not able to be measured directly with the equipment at hand, it is possible to infer its presence. In the image, the stomach is clearly distended but no signal is observed from inside it, whereas if the stomach was filled with food or liquid, this would appear as a signal at a longer relaxation time. This is a similar to how methane hydrates are measured using NMR (Kleinberg et al., 2003), where the absence of expected signal can be used to quantify their presence. However, due to the possible presence of other items in the stomach without an NMR signal, stones for example, this is clearly a suboptimal solution compared to measuring with equipment that can measure the necessary range of relaxations.

Identification of the plastic polymer type also appears to be feasible using NMR T_2 relaxation distributions. The plastic polymers show distinct NMR chemical and relaxation behaviour. However, measuring on real samples will present several challenges not investigated in this pilot study. Plastics that have been in the stomach for an extended period of time may degrade. This may alter their T_2 relaxation distributions, and this effect needs to be investigated in future studies. The presence of food or liquid in the stomach will also produce a measurable NMR signal. This will be at longer relaxation times than for the plastics, but the signal from food still will need to be deconvoluted from signal from plastic. Similarly, it is likely that two or more plastic polymer types will be present in the digestive system. In both these situations, multivariate analysis can be used to quantify the relative amounts of the different types of plastics or food and liquid in the stomach (Washburn and McCarney, 2018). Other types of prey objects, such as crab shells, appear to have relaxation times that are in the microsecond range, as tested examples produced no measurable signal. Objects like stone will produce no NMR signal in these types of measurements due to their chemical composition. Although quantification was not investigated in this study, NMR is well suited for quantitative measurements, as the signal linearly proportional to the amount of sample present.

Although the results of the study are promising, the technology is not immediately transferable for field use. It is not possible to transport standard MRI equipment to the field for measurement due to its size and power requirements. Whereas there has been made some strides in the development of portable hospital style MRI systems (Sheth et al., 2020), another, more practical solution would be the use of a single sided MRI sensor (Greer et al., 2019; Utsuzawa et al., 2021). In the case of one-sided NMR sensors, instead of the sample being placed inside the magnet, the magnetic field is projected laterally into the system under

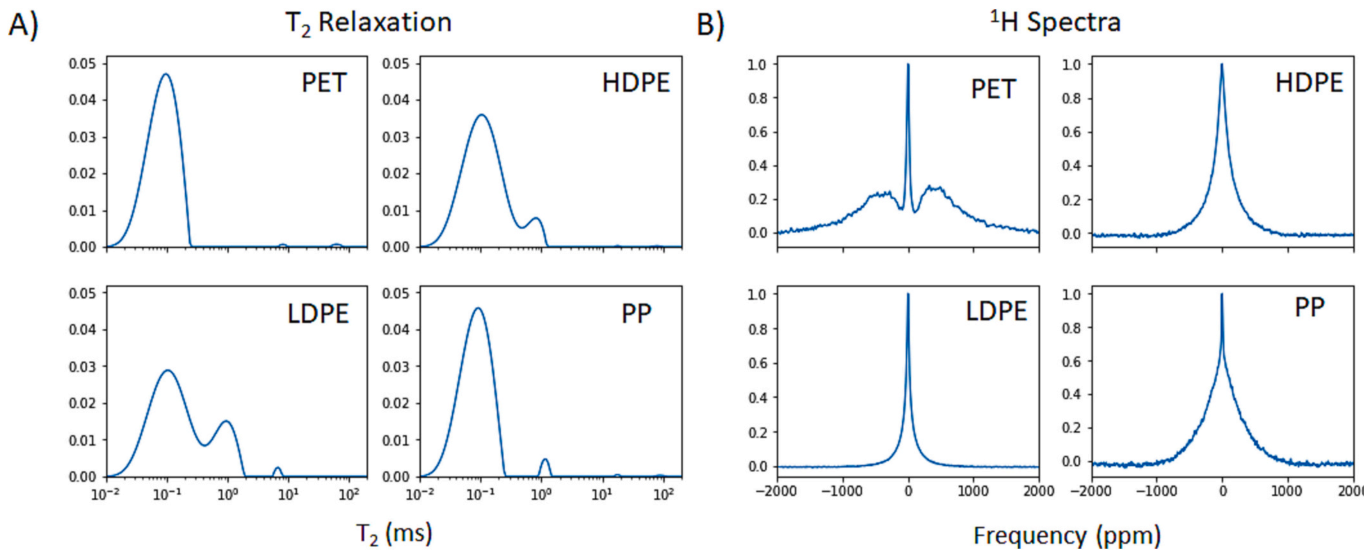

Fig. 4. Examples of A) T_2 relaxation time distribution and B) ^1H spectra for the four different plastic polymers.

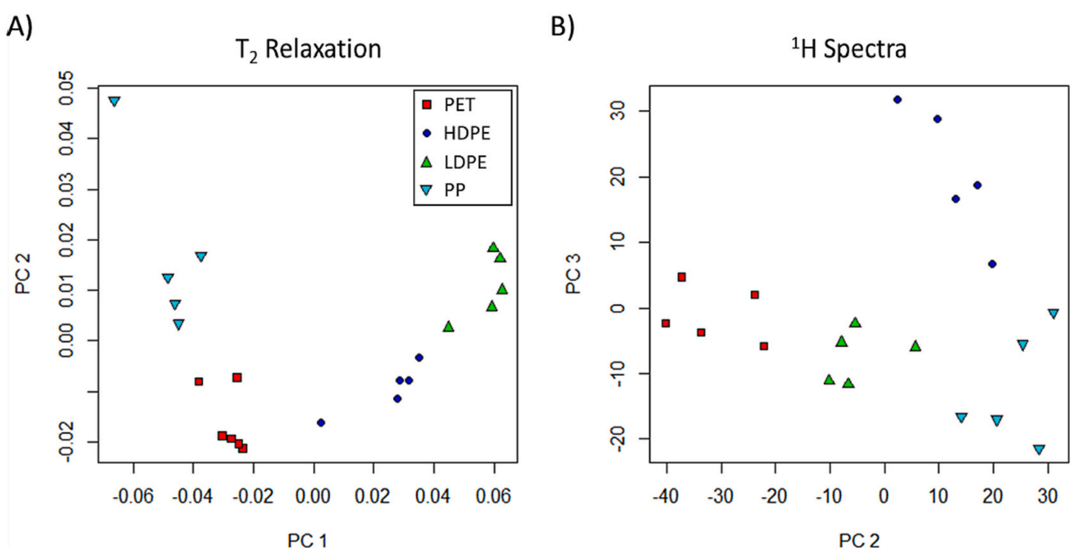

Fig. 5. Principal component analysis for A) T_2 relaxation distributions B) ^1H Spectra of different plastic polymers.

investigation. This makes them ideal for measuring large or awkward shaped samples, as well as enabling them to be made extremely portable if necessary. At the moment, there are no suitable commercial single sided sensors, but there exist a variety of research one-sided MRI sensors that could be adapted to measure on seabirds (Huang et al., 2019; Wald et al., 2019). The results here support the development of specialized custom MRI sensors for the measurement of plastic in the digestive system of seabirds and other marine wildlife. Once equipment optimized for imaging plastic in wildlife has been developed, validation studies would need to be performed in order to determine the best operational parameters, detection limits and perform the necessary calibration to convert the MRI signal to amount of plastic.

Despite the effort needed to develop an operable MRI field system, doing so would have numerous and overarching benefits. Non-invasive monitoring would enable significant improvements to be made to animal welfare while at the same time expanding monitoring capability. Although exact numbers are difficult to come by, thousands, or perhaps more, animal lives could be saved for the purpose of science, both in research on plastic pollution and other sorts of studies where dissection is often necessary, such as dietary studies. The rapid, non-invasive nature of MRI means more animals can be scanned during the course of field expeditions and money could also be saved by spending less time in the lab since no dissection or plastic extraction would be needed. The exact time of measurement will depend on the specifications of the developed MRI system, but it is likely to take a minute or two at most and quite possibly less. These improvements in efficiency will make a huge impact in terms of expanding biomonitoring, benefiting not only artic researchers, but for scientists all around the world.

5. Conclusions

Magnetic resonance imaging appears to be a promising, non-invasive technology for detecting plastic ingestion in wildlife. Inspection of basic MRI images enabled direct and indirect detection of the presence of plastic in the stomach. Analysis of common NMR measurements easily allowed the classification of plastic type. The non-invasive aspect enables the number of animals to be screened, which is particularly appealing for use in endangered species. However, further development in sensor equipment will be necessary before it can be applied in a field setting. If successful, the technology has the potential to greatly improve animal welfare while expanding biomonitoring capability in plastic pollution research.

CRediT authorship contribution statement

Kathryn E. Anderssen: Conceptualization, Methodology, Formal analysis, Data curation, Investigation, Resources, Writing – original draft. **Geir Wing Gabrielsen:** Resources, Methodology, Writing – review & editing, Funding acquisition. **Mathias Kranz:** Resources, Investigation, Writing – review & editing. **France Collard:** Resources, Investigation, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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